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13. ABSTRACT *(Maximum 200 words)*

My laboratory has generated a human mammary epithelial cell (HMEC) system for studies on normal and aberrant growth control mechanisms. As part of the goal to facilitate widespread use of HMEC, we have provided cells and cell culture expertise to over 150 laboratories worldwide.

To optimize use of this resource, we developed a complex database for storage and retrieval of information on the HMEC, and newsletters for communication among those using these HMEC. The purpose of this grant is to (1) upgrade and expand the features of this database, (2) ensure that all computer and written records can be readily understandable to others besides the PI, (3) create written histories of complicated cell lineages and enter them into the database, (4) develop an Email bulletin board to foster rapid, cooperative communication among laboratories using HMEC.

Most work on the database upgrade was completed in Year 1. Only minor additional changes have been necessary. For years 2 and 3, old records were entered into the database and work was begun on compiling cell histories and making written records more accessible. A web site on HMEC was launched and continues to be upgraded. This work is proceeding as planned, although it is clear that the history compilation will require more time than this granting period.

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[signature] 5/8/97

PI - Signature Date

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INTRODUCTION

My laboratory's research program has centered on the development and utilization of human mammary epithelial cell (HMEC) cultures. The overall goal of this work has been to generate human epithelial cell systems for studies on the normal mechanisms controlling proliferation and differentiation in human cells, and on how these normal processes may become altered as a result of immortal and malignant transformation. Included in this goal has been the desire to facilitate widespread use of human epithelial cells for molecular and biochemical studies. Therefore, we have endeavored to develop a system that is relatively easy to use, and can provide large quantities of well-characterized, uniform cell populations. We have already provided cells and cell culture expertise to over 150 laboratories worldwide, and new requests continue to be received.

During the past 19 years, we developed an HMEC bank which contains the following types of material: (1) primary cells (frozen as epithelial organoids or cell clumps) from reduction mammoplasties, tumors, non-tumor mastectomy tissue, benign tumors, and gynecomastias from nearly 200 individuals; (2) higher passage pools of single cells from the above tissue types; (3) cells from reduction mammoplasty specimens that have been exposed to benzo(a)pyrene and have acquired extended life in culture; (4) the immortally transformed 184A1 and 184B5 cell lines, including clonal isolates, and spontaneous and carcinogen induced variants of these lines; (5) malignantly transformed derivatives of 184A1 and 184B5. We have also generated three new immortally transformed cell lines (184AA2, 184AA3, 184AA4), and oncogene exposed (HPV16 E6, HPV16 E7, SV40T) derivatives of 184A1. Although these are not yet published, they have already been distributed.

The widespread usage of these many HMEC types necessitated development of an appropriate database for information storage and retrieval. A 4th Dimension Database was developed in 1987. One purpose of this grant is to upgrade and expand the features of this database, and to ensure that all computer and written records can be readily understandable to others besides the PI. The second major purpose of this grant is to set up an E-mail network among laboratories using HMEC. Originally, we distributed HMEC newsletters in order to foster rapid, cooperative communication among the groups using HMEC. The new technology of the Internet provides a better avenue for this function.

BODY

With reference to the specific aims of the proposal:

Update computer database

In Year One, as scheduled, we completed most of the work on the 4th Dimension Database upgrade. These changes have thus far have worked as intended, so that no further major changes appear necessary. Minor corrections and changes have been made on an ongoing basis. Further small changes will still need to be made before the end of this grant, and we anticipate that only minor changes will be required thereafter on an ongoing basis. Further work on documentation of use of the Database is still necessary.

Update information in the database

As scheduled for Years One and Two, the old records have been added to the database, and this work is completed.

We have continued checking the actual inventory in the freezers with what exists in computer records. Because this is difficult tedious work, it has moved slowly on an ongoing basis and is not yet completed.

Update written records on cell cultures

This has been a major effort for Year Three. The goal here has been to review old records to (1) rewrite when necessary to make intelligible; (2) distill histories which can be entered into the computer. In reviewing the amount of material that would benefit from update and clarification, it became obvious that this is going to be a long-term ongoing project, extending well beyond the time frame of this grant. I therefore chose to focus on the most important and worst written records - the three experiments in which

normal HMEC were exposed to a chemical carcinogen, from which extended life cultures and immortally transformed cell lines were obtained. Last year I completed the worst records [the 184 C (cross) series] and began the 184 B series records. This year I have completed the records of the derivations and early histories of the 184 B and the 184 A series. This again was very difficult and time consuming, and a small amount of information could not be deciphered. It was also quite rewarding, since new discoveries in our laboratory on the nature of immortal transformation in HMEC made it essential that I be able to know in greater detail the history of the origins of these immortal cell lines. I rewrote the records in a way that others in the lab attested to being comprehensible. These distilled histories were added to the database (see appendix A and B). We have also been more careful to keep generally intelligible records and write up distilled histories on an ongoing basis. We are in the process of completing the distilled histories for our three new immortal cell lines, 184AA2, 184AA3, and 184AA4.

With reference to records that still need to be entered into the database, these include:
(a) Histories of derivatives of 184A1 and 184B5, such as nutritional variants and oncogene exposed cells. The ongoing records for clonal derivatives of these lines also need to be distilled for entry into the database.
(b) Primary tissues. There are around 150 specimens for which data on specimen history and original growth capacity needs to be entered.
(c) Finite lifespan epithelial cultures from reduction mammoplasties, mastectomies, and benign tumors. There are around 25 specimens which have been grown in long-term culture. Relevant data on the growth capacity of each cell batch, and experimental studies performed on these specimens needs to be entered.
(d) Fibroblast cultures from the same specimens as the HMEC. Growth capacity for these cells needs to be entered.
For most of these cultures, the original records do not need to be rewritten; what is required is distillation of the essential information into the database so that others can readily access the relevant information.

Another aspect of this work is to go through our 18 notebook files of written records to ensure that records are filed in the appropriate places (many were not). This work is nearly completed and should be complete by the end of this grant. No major problems have been encountered.

Set up an Email network and communications network

This has been the secondary focus of our work in Year Three. My initial goal was to post on the Internet most of the information that I now routinely need to send to or talk about with other investigators. Last year we launched our web site (http://www.lbl.gov/~mrgs). This year I have continued to work with a student assistant to refine our Email network. We have:
(a) corrected and updated some of the information posted last year
(b) added new Procedure sections
(c) refined and completed our Investigator List section
(d) refined and completed our legal forms section
With these additions, the web site now contains all the information I need to provide to other investigators who are interested in obtaining HMEC from me, or who want information on HMEC derivation and culture. It has been a very major benefit for me to be able to refer all queries to my web site. I no longer need to send information on an ongoing basis, and other investigators now have a better idea of the HMEC available before discussing their scientific needs with me.

In addition, the student assistant worked with the LBNL computer specialists to make the web site at least partly interactive. A group mailing list was developed such that anyone on the mailing list can send and respond to queries from anyone else on the mailing list. An announcement of this feature of the network was sent out to the mailing list in February (see Appendix C). With reference to the list of participants in the Email network, Appendix C also indicates the approximately 90 people to whom this original announcement was sent. This list needed some corrections and currently needs updating.

For the future, there is still much more information I would like to post on the web site about HMEC procedures, and unpublished data from my lab and others that would be generally useful to those working with HMEC. I also want to keep current the information presented in the review of the HMEC system on the web site.

General provision of HMEC and information about HMEC

Although this is not a stated task of this grant, I realize that a significant percentage of my time is engaged in assisting other investigators with their usage of HMEC. I believe this is a very cost effective situation for general research in human breast cell biology, and a role I am comfortable doing. I receive around 1-4 requests/queries per week about HMEC via Email, fax, or telephone. In the past year, we sent 26 shipments of cells to 21 different investigators. I am aware of around 20 additional laboratories using these cells on an ongoing basis, plus there are many groups using these cells with which I am not in ongoing contact. I also respond to many queries on HMEC use, biology, and problems with cell culture, from a variety of investigators, some of whom have obtained HMEC from Clonetics or cell repositories (Coriell, ATCC), or are interested in beginning studies with HMEC. This ongoing interest reminds me of the need to ensure that our cells, and information about them, can be readily accessible. It is difficult to predict what will be needed in the future, however, the present suggests that getting my HMEC records and database in user-friendly shape is a task I should continue.

CONCLUSIONS

The work has been proceeding mainly as planned, with the exception of the realization that reviewing and redoing the cell histories is a much more formidable task than originally anticipated. The Database has been updated, old records have been entered, the reordering of filed records has proceeded well, and the Email bulletin board has been successfully launched and now functions as a major resource for myself and others. The two slow aspects of the project are the checking of the freezer inventory, and the compilation of the histories. Since I realized that the work on histories will continue well beyond Year Three, I have focused on those histories for which there is the most urgent need for review and compilation - the experiments that led to our Extended Life cultures and immortally transformed cell lines. I have also learned the importance of keeping good ongoing histories.

APPENDIX A

The following information is entered under the history for 184, exposed to benzo(a)pyrene

184 Aleph (א)

This was the first in the series of three experiments in which primary cultures of specimen 184 growing in MM were exposed to the chemical carcinogen benzo(a)pyrene (BaP) in the hope of obtaining immortally transformed cell lines. This series was called "aleph" (א) after the first letter of the Hebrew alphabet (alephbet). Being the first such experiment, it did not follow a fixed protocol. In retropsect, I would say that the goal to obtain immortally transformed cells superceded the usual scientific method of fixed protocols for testing variables and approaches. On the positive side, this experiment did lead to the immortally transformed line 184A1. On the down side, approach and record keeping left a lot to be desired.

Record keeping for this experiment preceded our standardized record sheets and protocols. There was no attempt to separate cells based on lineages, media, or treatment. Notes were written by Kristy Venstrum, myself, and Annie Pang. Kristy did most of the culture in 202. Kristy and my notes are generally good - but we often didn't indicate medium (where not critical for ID), or exactly how many dishes cells were seeded into, or what dishes (#, size) were trypsinized. Annie, in addition to almost never indicating medium (even when dishes were otherwise identical), also frequently omited such useful ID information as experimental condition (A, B, C, D), passage number, and lists passage date sometimes as the date of the last PT. Since the lineages were not too complex, I could usually, though not always, figure out what was done. I went over all these records and re-wrote them to be more legible and to separate out cells grown in different media, and different lineages. These are filed along with the original notes in the beginning of the 184A1 notebook.

The experiment was begun on 2/10/82 by seeding 1 ampoule of 184 organoids into 9 T-25s and 4-35s with MM, and 4 T-25 with MCDB 202. Flasks/dishes C,C',D,D' in MM received 1μg/ml BaP two times for 24 hr each, on:
day 7 (2/16) 24 prior to PT2
day 16 (2/25) 1 day after PT6 and 1 day prior to PT7
Flasks A,A',B,B' (controls) received DMSO at the same times.. Flask E was given to Linda Hayashi.

The B and C flasks were fed with MM plus 200μM Fatty Acids (FA) which at the time I thought might promote transformtion.

On a personal historical note, while 184Aa was transforming into 184A1, I was dealing with my best friend dying of Hodgkins disease (lymphoma) in my home (on 5/13/82), apparantly being away for several weeks after that (no MS notes from 5/19-6/10 when some crucial dishes of earliest 184A1 were apparantly discarded) and moving our whole cell culture operation from the Peralta Cancer Research Institute to Bldg 934 under less than optimal physical and emotional conditions (first freezedown at Bldg 934 recorded 7/3/82 - AA000 - a very early 184A1).

In summary:

<u>184א in MM</u>
There were minor differences between control and BaP treated cells, or ±FA, other than one Extended Life culture, 184Aa, which ultimately gave rise to the immortal line, 184A1. See also Figure 3 from EMail review for more information. We subsequently found out that both 184Aa and 184A1 have mutations in both alleles of the p16 gene. 184Aa first appeared at p5 of PT of flask D as a single morphologically distinct patch growing in what I refered to as the "pimple" pattern - swirling around a pointy center. Based on this and the p16 mutations, 184Aa is presumably clonal. 184A1 first appeared in the 184Aa culture at p9, readily distinguishable by its more rapid growth, growth in singlets vs. patches at low density, and more refractile appearance. Something I can not quantitate told me on first sight that these were immortal cells. Based on its few specific karyotypic abnormalities, 184A1 also is clonal in origin.

<u>BaP Treated</u> (flasks C,C',D,D')
PT1: day 7 (2/16), seed cells from D (1st BaP 4 hr later)
freezedown from C flasks (**671AC**)

PT2: day 8 (2/17), good regrowth, seed cells from C and D (35mm dishes) (day after 1st BaP treatment of 0.67µg/ml)
freezedowns from C flasks (**681AC**), from D flasks (**680AC**)
PT3: day 10 (2/19), good regrowth, seed cells from C and D
freezedowns from C flasks (**703AC**), from D flasks (**684AC**)
PT4: day 12 (2/21), good regrowth; cells discarded
PT5: day 14 (2/23), good regrowth; cells discarded
PT6: day 15 (2/24), still look good, a little ridging; cells discarded (2nd BaP next day)
PT7: day 17 (2/26) good, a bit heavy, some beginning signs of slowing down. Seed cells, some into MCDB 202
PT8: day 19 (2/28) cells very confluent; discard
PT9: day 21 (3/2) cft areas, not so good in hemacytometer, seed cells from C and D
PT10-C: day 26 (3/7); discard
PT11-C: day 30 (3/11), heavy cft with growth at edges, seed cells from C
PT12-C: day 31 (3/12); ?discard
PT13-C: day 35 (3/16), heavy cft areas with growth at edges; ?discard
PT14-C: day 41 (3/22), all aged, not much growth; ?discard
PT15-C: day 50 (3/31); ?discard
PT16-C: day 57 (4/7), rseveral decent but not great growing areas; ?discard;
PT10-D: day 30 (3/11), growing and fading, seed cells from D
PT11-D: day 35 (3/16), growing and aged; ?discard
PT12-D: day 41 (3/22), all aged, not much growth; ?discard
PT13-D: day 50 (3/31); ?discard
PT14-D: day 57 (4/7), rare areas of growth, 1 patch heavy, rare mitises in center; ?discard; ?D listed as yeast nd discarded
on day 86 (5/6) flask C was recorded as so-so aging and discarded

PT2 (2/17):
p2 grown ±CT; good growth
p3 mostly good
p4 OK-good, growing areas and older
p5 swirly aged-growing
p6 swirly aged and some growing
p7 mostly aged, yukky, ? any growth
END

PT9 (3/2) C and D
p2 OK-good
p3 good growing
p4 good growing- aged-growing
p5 aged, some aged swirly growing, ?growing (1 dish gives rise to 184Aa - see separate listing)
END

PT10 (3/11) C and D
p2 so-so swirly -active growing
p3 mostly good growing, growing among aging
p4 swirling, heavy yukky
END

PT16 (4/7) C (on UV FB)
p2 aging and growing, yukky
p3 large patch starting to age (BPC) > still growing after multiple DTs > almost cft
p4 continuation of BPC above, becomes subcft-cft so-so, decent growing areas
p5 BPC continues, so-so aging to cft
this lineage seems to have been eventually discarded due to "left on bench" and not growing
END

Controls (flasks A,A',B,B')
PT1: day 7 (2/16), seed cells fromB (1st DMSO 4 hr later)
freezedown from A flasks (**670AC**)

PT2: day 8 (2/17), good regrowth, seed cells from A and B(35mm dishes) (day after 1st DMSO treatment)
freezedowns from A flasks (**678AC**), from B flasks (**702AC**)
PT3: day 10 (2/19); good regrowth
freezedowns from A flasks (**683AC**), from B flasks (**679AC**); (good regrowth)
PT4: day 12 (2/21), good regrowth, a little slowing down; cells discarded
PT5: day 14 (2/23), good regrowth; cells discarded
PT6: day 15 (2/24), still look good, a little ridging; cells discarded; 2nd DMSO next day
PT7: day 17 (2/26) good, a bit heavy, some beginning signs of slowing down. Seed cells from A and B
PT8: day 19 (2/28) cells very confluent; discard
PT9: day 21 (3/2), overly cft but good cells, seed cells from A and B
flask B lost on day 22 to yeast (no record of ' flasks after day 14)
PT10: day 30 (3/11), growing and fading, seed cells from A
PT11: day 35 (3/16), growing and aged; discard
PT12: day 41 (3/22), all aged, not much growth; discard
PT13: day 50 (3/31); discard
PT14: day 57 (4/7), rare areas of possibly growing cells; ?discard
on day 86 (5/6) flask A was recorded as so-so aging and discarded

PT2 (2/17):
p2 grown ±CT; good
p3 to confluence
p4 unrecorded, including split
p5 flat bigger cells not perky or growing
p6 old large and pockets of reasonably good growing
p7 mostly aged, yukky, ? any growth
END

PT7 (2/26) A+CT
p2 good; comtaminated:
END

PT9 (3/2) A
p2 OK-good
p3 good
p4 so-so aged-growing
p5 nothing vaguely growing
END

PT10 (3/11) A
p2 so-so, some growing
p3 aged
END

The following information is entered under the history for 184Aa (184 ELAa)

184Aa: (in the original records, this is called BPD)
This was derived from **PT9** on day 21 (3/2), from flask D of the 184ϰ experiment (see listing for 184ϰ).
3/7 p2 3/2 subcft growing > 2-60
3/12 p3 3/7 subcft good growing > 2-60:
3/19 p4 3/12 subcft so-so aged growing > 3-60

p5 3/19:
4/7 I note 1-60 has one heavy patch; other 2-60 have "nothing vaguely growing" and are discarded
4/19 good growing patch with swirls, PT1 >1-60 , 2-35
4/27 patch regrowing well PT2 >2-60, 3-35+
5/4 swirly aged cells near cft (presumptive PT3)
5/7 cft PT4 > ?
5/10 heavy cft PT5 > discard
5/13 1-60 PTed 5x yeast > discard. End

p6 lineages:
p6 4/19:
4/27 subcft-cft tightly packed ridged, still mitoses PT1 1-60 > 2-60 and colonies on UV FB
4/28 cft heavy with blank areas 2-35 > freezedown (**829AC**)
5/4 1-60 PT2 and discard (arggh)
5/7 cft PT3 > ?
5/7 colony dish good growing > 2-35+
5/10 heavy cft PT4 > discard
5/13 heavy cft PT5 > discard
6/6 KV notes good very cft with many ridges
6/23 very cft, yeast > discard. End
p6 4/27:
5/7 subcft-cft swirly aged and growing > 60s
5/7 2-60 > freezedown (**848AC**)
5/11 1-60 yeast > discard. End

p7 lineages:
p7 4/27:
5/7 mostly cft aged-growing swirls > 2 T75, 2-60, 2-35+ and some into MCDB 202
5/7 > freezedown (**849AC**)
5/14 colonies nearly cft, growing and aged ridging > 1-60, 2-35, colonies, freezedown (**856AC**)
p7 5/7
5/10 ?dishes not fed > discard
5/17 mostly cft aging-ridged and a little growing > 1-60, 1-35, colonies
5/28 very cft patches, OK > 2 T75
6/3 cells (no amount or description) indicated as discarded by AP.
6/6 KV notes good cft

p8 lineages:
p8 5/7
5/17 growing and aging-ridged > 2-60, colonies
5/21 from colonies, so-so growing in patched > 1-60, 1-35
5/27 2T75 subcft-cft growing in patches, swirling > 2 T75, 2-60, freezedown (**872AC**)
6/3 2-60, 2-35 so-so cft cells > discarded (arghh, no reason given - I was away)
p8 5/14
6/6 KV notes OK large growing patches
6/14 KV notes possible appearance of 184A1; see 184A1 for further records of this lineage
p8 5/17
6/6 KV notes OK-good med growing patches
p8 5/28
6/6 KV notes OK small slowly growing patches

p9 lineages:

p9 5/17
6/6 KV notes subcft med-good growing patches
6/14 MS notes appearance of 184A1; see 184A1 for further records of this lineage
p9 5/27
6/6 KV notes subcft -cft small slowly growing patches
6/14 MS notes appearance of 184A1; see 184A1 for further records of this lineage
Since 184A1 grew much faster than 184Aa, and took over the cultures, this ends records for 184Aa

The following information is entered under the history for 184A1

184A1
This was the first in the series of three experiments in which primary cultures of specimen 184 growing in MM were exposed to the chemical carcinogen benzo(a)pyrene (BaP) in the hope of obtaining immortally transformed cell lines. This series was called "aleph" (א) after the first letter of the Hebrew alphabet (alephbet). Being the first such experiment, it did not follow a fixed protocol. In retropsect, I would say that the goal to obtain immortally transformed cells superceded the usual scientific method of fixed protocols for testing variables and approaches. On the positive side, this experiment did lead to the immortally transformed line 184A1. On the down side, approach and record keeping left a lot to be desired.

Record keeping for this experiment preceded our standardized record sheets and protocols. There was no attempt to separate cells based on lineages, media, or treatment. Notes were written by Kristy Venstrum, myself, and Annie Pang. Kristy did most of the culture in 202. Kristy and my notes are generally good, Annie's were sometimes inadequate or inaccurate. I went over all these records and re-wrote them to be more legible and to separate out cells grrown in different media, and different lineages. These are filed along with the original notes in the beginning of the 184A1 notebook.

184A1 was obtained from **184Aa (ELAa)** which came from **PT9** on day 21 of primary flask D from the experiment 184א, which had received 1µg/ml BaP two times for 24 hr each.. See 184 ELAa for the history of the extended life culture prior to the appearance of 184A1, see 184א for details of the experimental protocol.. Fortunatley, the emergence of both 184Aa and 184A1 was fairly straightforward. Although there were some PTs invovled, in general, passage numbers can be considered linear from emergence.

My first recollection of 184A1 is noting aggressively growing small singlet cells in flasks of slowly growing flat patchy p9 184Aa. I assumed immediately that these cells were immortal, for reasons I can't specifically quantitate - although in this case (unlike 184B5) the cells were showing good growth..

The ELAa direct lineage leading to 184A1 was: **p5 3/19 PT1 > p6 4/19 PT1 > p7 4/27 PT1** (**849AC** 5/7 184Aa)(**856AC** 5/14 184Aa but could contain 184A1)) > **p8 5/7** and p8 5/14 (**872AC** this could contain 184A1) **> p9 5/17** and **p9 5/27** (**902AC**, **903AC**).

Earliest notes:
p8 5/14
6/6 KV notes OK large growing patches
6/14 KV notes "good cft ep patches with FBs, some less densely packed areas growing out"
This could be signs of 184A1 at p8.
6/20 MS notes "most areas aged but one large good growing area"; this was subcultured and then lost to yeast

p9 5/17
6/6 KV notes subcft med-good growing patches
6/14 MS notes "cells have different morphology. Squarer growing more as singlets rather than patches of atteched cells"; KV notes "good less densely packed cft and dividing ep (not patchy anymore - its a monolayer!)"
1-60 > T25, 4-35+, colonies, methocel
1-60 > 2T25 each MM and MCDB 202
6/24 2-60 subcft so-so PT2 > T75, 3-35+ and T25, 3-25+ in MCDB 202
p9 5/21
6/14 KV notes "good cft patches with many mitoses (small octagonal cells)"
This culture was unfortunately discarded (had FB too)
p9 5/27
6/6 KV notes subcft -cft small slowly growing patches
6/14 MS notes "same densely packed slowly dividing ep patches but areas of less dense but vigourously mitotic cells coming off the patches. Many cells lifting off near these growing areas"
6/23 2 T75 good-OK patchy cft > freezedowns (**902AC**, **903AC**)
6/24 MS "subcft-cft some heavy starting to ridge, other growing areas"
1-60 > T75, 3-35+ and T25, 3-35+ in MCDB 202
?1-60 > 2 T75

p10 6/24 (from p9 5/17 PT2 and/or p9 5/27)

7/2 MS "look good, med-subcft with single cell growth and ridging where more cft" >3T75, T25
7/3 2 T75 > freezedowns per each flask (**AA000**, **AA001**) (first Bldg 934 freezedowns!)

P11 7/2 (from above)
7/12 good cft monolayer, small refractile cells 1 T75 > freezedown (**AA005**)
2 T75 > freezedown (**AA004**) , 2 T75, 4 T25, 4-35+

Further original lineages in MM looks pretty straightforward from here. Freezedowns follow from this, e.g., AA012, AA013, AA014, AA017, AA018, AA022, AA064, AA073, AA087, AA108, AA114, AA124, AA148, AA166, AA205, AA220

APPENDIX B

The following information is entered under the history for 184, exposed to benzo(a)pyrene

184 Birdie (v) in MM

This was the third in the series of three experiments in which primary cultures of specimen 184 growing in MM were exposed to the chemical carcinogen benzo(a)pyrene (BaP) in the hope of obtaining immortally transformed cell lines. It followed the same general protocol as the first two experiments ("aleph" and "cross"). This series was called "birdie" to balance the previous two names (ethnic/religious symbols) with a nature symbol (of which I am very fond).

Our record keeping for this experiment generally followed the previously established protocol in terms of using the standardized sheets and separation by different medium. However, separate lineages were not separated (e.g., the different EL cultures; treated vs. controls). I went over all these records and re-wrote them to be more legible and to separate out the different lineages. These are filed along with the original notes in the beginning of the 184B5 notebook.

The experiment was begun on 5/4/83 by seeding 1 ampoule of 184 organoids into 4 T-25 with MM, and 4 T-25 with MCDB 170. Flasks C,D in MM received 1μg/ml BaP three times for 24 hr each, on:
day 7 (5/10) 24 prior to PT1
day 10 (5/13) immediately after PT2 and 24 hr prior to PT3, which generated the BP1 subculture
day 23 (5/26) 24 hr prior to PT7, which generated the BP3 subculture
Flasks A,B (controls) received DMSO at the same times

The 4 flasks in MCDB 170 (also called A,B,C,D) were not part of this experimental protocol and records for them are found in the 184 records book under 184 birdie (v).

In summary:

184v in MM
BaP treated cells produced two morphologically distinct Extended Life cultures: Be, and Bf, Bg, Bh. 184Be ultimately gave rise to the immortal line 184B5. Based on its specific karyotypic abnormalities, 184B5 is clonal in origin.
See also histories under individual EL listings (184 ELBe, 184 ELBf, 184 ELBg, 184 ELBh) for specific information on each EL culture for which there are EL freezedowns. See below for others. See also Figure 3 from EMail review for more information.

BaP Treated (flasks C,D)
PT1: day 8 (5/11) (1st BaP 24 hr prior)
cell frozen (**AA302**)
PT2: day 9 (5/12) followed by 2nd BaP treatment
cells frozen (**AA308**)
PT3: day 10 (5/13) somewhat sickly, growing, subcft. > **BP1**
PT4: day 14 (5/17) so-so mitoses and sickly
cells frozen (**AA314**)
PT5: day 17 (5/20) some good regr. and sickly; aged > **BP2**
PT6: day 20 (5/23) almost cft heavy areas
cells frozen (**AA326**)
PT7: day 24 (5/27) (3rd BaP 24 hr prior) mostly cft aging and some growing > **BP3**
PT8: day 27 (5/30) mostly cft, heavy in areas, aging and growing; discard
PT9: day 31 (6/3) heavy cft and growing areas; flask C only hereafter > **BP4**
PT10: day 34 (6/6) cft OK rare mitotic areas; discard
PT11: day 37 (6/9) good mitotic medium size 2 patches > **BP5**
PT12: day 43 (6/15) 2 large patches OK > **BP6**
PT13: day 49 (6/21) growing area, ridging and OK > **BP7**
PT14: day 56 (6/28) large aging/growing/ridging patch >**BP8**
PT15: day 63 (7/5) heavy aging recovering from acid (?), mitoses at edges; discard

PT16: day 65 (7/7) reasonable regrowth > **BP9**
Flask C and BP9 were contaminated with yeast on day 66 (7/8) and discarded

BP1:
This was PT3 on day 10
p2: cft - over cft
p3: OK cft
p4: few growing cft foci, mostly so-so cft; 1 large patch senescing/ridging PTed x3
p5: mixed; no growth to patchy good growing and cft aging
p6: contaminated or no growth
END

BP2:
This was PT5 on day 17
p2: good growing and aging
p3: good growing areas, rest so-so
p4: subcft aging and ridging, good regrowth after PT; swirling aging with areas of good growing cells between
p5: mixed; elongated swirling with nests of cuboidal growing cells, patchy growing with aging; dish with good growing area and yeast
p6: looks like most got contaminated so can't fully say if anything would have maintained growth longer
END

BP3:
This was PT7 on day 24
p2: mostly cft good growing and some aging, so-so
p3: good growing areas
p4: looks like got contaminated or no records (maybe discarded for no growth) so can't fully say
END

BP4:
This was PT9 on day 31
p2: good so-so cft
p3: looks like got contaminated or discarded (? for no growth) so can't fully say
END

See under EL records (184 ELBe, 184 ELBf, 184 ELBg 184 ELBh) for BP5, BP6, BP7, BP8

Controls (flasks A,B)
PT1: day 8 (5/11) (1st DMSO 24 hr prior)
cells frozen (**AA303**)
PT2: day 9 (5/12) followed by 2nd DMSO treatment
cells frozen (**AA307**)
PT3: day 10 (5/13) good gr. > **C1**
PT4: day 13 (5/16) heavy cft
cells frozen (**AA312**)
PT5: day 15 (5/18) good cft- OK
cells frozen (**AA318**)
PT6: day 17 (5/20) good regr. and some aged; discard
PT7: day 20 (5/23) subcft-cft OK
cells frozen (**AA325**)
PT8: day 24 (5/27) (3rd DMSO 24 hr prior) mostly cft aging and some growing > **C3**
PT9: day 27 (5/30) mostly cft aging and some growing; discard
PT10: day 31 (6/3) heavy cft and growing areas; flask B only hereafter > **C4**
PT11: day 34 (6/6) very cft areas; discard
PT12: day 37 (6/9) so-so cft- very cft > **C5**
PT13: day 41 (6/13) so-so cft. 1 large area; discard
PT14: day 44 (6/16) OK-so-so large patch; discard

PT15: day 49 (6/21) growing, crowded, ridged > **C7**
PT16: day 56 (6/28) old, aging, ridging; discard
No further records of fate of flask B (flask A listed as discarded on 6/28)

C1:
This was from PT3 on day 10
p2: good-OK
p3: good cft
p4: so-so cft, few gr. areas are cft
p5: no growth
END

C3:
This was from PT8 on day 24
p2: mostly cft good growing, some aging so-so
p3: no growth
END

C4:
This was from PT10 on day 31
p2: good so-so cft
p3: no growth
END

C5:
This was from PT12 on day 37
p2: no growth
END

C7:
This was from PT15 on day 49
p2: no growth
END

The following information is entered under the histories for 184 ElBe, ELBf, ELBg, and ELBh

ELBe:
This was derived from **BP5** which was from PT11 on day 37, from flask C, recorded as good mitotic medium size 2 patches
p2: 3-35s were seeded, in one there were 2 good growing patches, heavily mitotic, with little aging, ridging (A); in one good nearly cft patch (B); in the third there was no growth
p3A: good growth
cells frozen (**AA357**, OK-cft)(**AA362**, cft-heavy-cft good growing and cft aging, swirling, from p2 after 1 PT)
p3B: good-OK growth
cells frozen (**AA361**, almost cft good growing areas and older aging, swirling)(**AA370**, mostly good cft-subcft few swirls, from p2 after 1 PT)
p4A: good growing with some aging swirling
cells frozen (**AA366**, med-cft as above)(**AA369**, cft-subcft, from AA362 lineage)
p4B: good-OK growing and some aging swirls
cells frozen (**AA371**, subcft-cft as above from AA361 lineage)
p5A: good growing and swirling aging. A variety of lineages generated. For detail see 184B5 history (MM and MCDB 170)
p5B: discarded - no record why. End of B lineage
p6: many good areas of growth, some so-so, some no growth. 184B5 shows it face for the first time in 1-35 dish p6 7/18. It's convoluted history from this point is under 184B5. The rest of this is ELBe that is only EL. One dish is reported to have a "hyperplasia" growing patch, but it got contaminated after a PT.
p7: some so-so growth
Hard to say after this point as the records seem to be following the emergent 184B5. Looks like a lot of discarded dishes at p6 and p7, so I suspect ELBe doesn't go too much further here (in MM)
END

ELBf:
This was derived from **BP6** which was from PT12 on day 43, from flask C, recorded as 2 large patches OK
p2: patches mostly cft, growing, swirling, aging
p3: good growth
cells frozen (**AA368**, OK-cft)
p4: good growing and also aging ridging cft flat
p5: largely aging with pockets of growth; some small good gr patches. Various PTs. From variety of dishes would say this is a mixed passage - aging of what was growing well to this point plus new pockets of growth.
cells frozen (**AA439**, after 5 PTs, widespread good regrowth)
p6: the pockets of growth continue, good growth and I first note the appearance of the "hyperplasia" morphology (see Email review and original 1985 paper) with good growth.
cells frozen (**AA440**, after 2 PTs, good regrowth of hyperplasia areas)(**AA442** good subcft-cft growing hyperplasia)(**AA444**, heavy cft in areas and sparser growing areas)
p7: mostly good growth and some aging where heavier, some vacuolated
cells frozen (**AA448**, **AA453** cft OK)
p8: growing and aging, vacuolated; a mixed passage
cells frozen (**AA484**, after 1 PT, good regrowing areas, heavy patches)
p9: most discarded (?no growth) or contaminated; still some dishes with mixed growing and aging, vacuolated.
cells frozen (**AA489**, mixed growing and aging)
p10: mostly no growth, discarded. There was a CO2 problem around the end here which may have sent cells to an earlier demise
END (for MM)

ELBf switched to MCDB 170
A variety of dishes were switched from MM to 170 at p8 and p9; then switched dishes were also subcultured. Since not all switches were recorded there is some mystery here as to exact origins of some dishes.
p8: good growing and aging
p9: mixed, good growing and aging
cells frozen (**AA498**, OK cft - switched at p8)
p10: still some good growth of patches
cells frozen (**AA494**, cft patches good -switched at p9)
p11: still some good growth of patches
p12: whatever left discarded. Most of previous passages discarded too.
END

ELBg:
This was derived from **BP7** which was from PT13 on day 49, from flask C, recorded as growing area, ridging and OK
p2:subcft-cft, growing, swirling, aging
p3: good growing and some swirling aging
cells frozen (**AA367**, good growing cft)
p4: aging with pockets of growing; good regrowth after PTs
cells frozen (**AA375**, OK-so-so cft-subcft)
p5: largely swirling aging with pockets of good growth; good regrowth after PTs.
p6: largely aging with pockets of good growth and I first note the appearance of the "hyperplasia" morphology (see Email review and original 1985 paper) with good growth, so dishes eventually have this good growth
cells frozen (**AA432**, after 2 PTs, OK heavy cft areas)(**AA441**, after 3 PTs good regrowth from patches left)
p7: mixed growing and aging
cells frozen (**AA438**, med-cft aging and growing)(**AA443**, subcft mixed good growing and aging)(**AA451**, subcft-cft heavy patches good)
p8: no good growth
END (for MM)

ELBg switched to MCDB 170
A variety of dishes were switched from MM to 170 at p7 and p8; nothing much grew and there were no freezedowns
END

ELBh:
This was derived from **BP8** which was from PT14 on day 56, from flask C, recorded as large aging/growing/ridging patch
p2: subcft-cft, good growing, ridging, aging
cells frozen (**AA356**, OK cft)
p3: good growing and some swirling aging
cells frozen (**AA364**, good growing and aging swirling subcft)
p4: mostly aging with pockets of OK growing
cells frozen (**AA372**, so-so mostly aging OK growing areas subcft-cft)
p5: largely swirling aging with pockets of good growth; many PTs
p6: largely aging with pockets of good growth; many PTs, and I first note the appearance of the "hyperplasia" morphology (see Email review and original 1985 paper) with good growth, so dishes eventually have this good growth
p7: mostly good growth
cells frozen (AA449, subcft-cft growing and older)(**AA467**, med-subcft growing-OK)
p8: growing and aging
cells frozen (**AA452**, subcft good)(**AA455**, cft aging and growing)
p9: aging and growing areas
p10: no growth recorded. There was a CO2 problem around the end here which may have sent cells to an earlier demise
END (for MM)

ELBh switched to MCDB 170
A variety of dishes were switched from MM to 170 at p7-10; then switched dishes were also subcultured. Since not all switches were recorded there is some mystery here as to exact origins of some dishes. There were no freezedowns.
END

The following information is entered under the history for 184B5

184B5

This was derived from the third in the series of three experiments in which primary cultures of specimen 184 growing in MM were exposed to the chemical carcinogen benzo(a)pyrene (BaP) in the hope of obtaining immortally transformed cell lines. It followed the same general protocol as the first two experiments ("aleph" and "cross"). This series was called "birdie" to balance the previous two names (ethnic/religious symbols) with a nature symbol (of which I am very fond).

Our record keeping for this experiment generally followed the previously established protocol in terms of using the standardized sheets and separation by medium used. However, separate lineages were not separated (e.g., the different EL cultures; treated vs. controls). I went over all these records and re-wrote them to be more legible and to separate out the different lineages. These are filed along with the original notes in the beginning of the 184B5 notebook.

184B5 was obtained from **ELBe** which came from **BP5** which was from PT11 on day 37 of primary dish C, which had received 1µg/ml BaP three times for 24 hr each. It was recorded to have two good mitotic medium size patches on day 37. The control flask B on that date was recorded as so-so cft-very cft and had PT12 which gave C5, which had no growth in p2. The 2 patches on flask C were again noted on day 43 for PT12 and BP6. See ELBe for the history of the extended life culture prior to the appearance of 184B5. The earliest history of 184B5 is rather convoluted due to (1) multiple PTs of multiple dishes, leading to complicated lineages and an inability to assign exact passage numbers and growth records to the earliest 184B5; (2) a yeast contamination of early 184B5, which I recall treating very aggressively, further complicating lineages and passage numbers.

My first recollection of 184B5 is spotting a small sickly patch of tightly packed cells in one corner of 1-35 mm dish. Although the cells looked less than wonderful or lively (i.e., not particularly mitotic) I was struck immediately by some aspect of this patch which told me that these cells were immortal. I wish I could say precisely what I was registering, but I can't. However, as a consequence, I went to great lengths to maintain these cells in face of the above mentioned yeast infection and their slow growth, whereas I did not do the same for the other many EL patches that showed up in the course of these three experiments.

ELBe at p5 had mixed growth, from good growing areas to so-so and aging. p6 also had mixed growth and one note of the "hyperplasia" growth. On 7/18 2-60s of a **p5 7/13** culture (good swirling cft-subcft) were subcultured to **p6 7/18** 3-60 and 3-35. This is the same lineage from which the ELBe freezedowns **AA369** (p5 from p4 7/8 frozen on 7/13) and **AA362** (p4 from p3 7/1 frozen on 7/8) were obtained.

It was noted that 2 60s and 1 35 of p6 7/18 were discarded due to contamination. I don't know the fate of the remaining 1-60 and 1-35 beyond the 1-35 described below.

On 8/29 I first noted the 184B5 patch in 1-35 of **p6 7/18**. Dish got PT1
On 9/4 I noted an OK growing patch. PT2 generated 1-35 of p7 9/4

p7 9/4 1-35 lineages: this dish was subjected to multiple PTs on
9/23 (PT1) > p8 9/23 2-35 (subcft so-so with some areas of growth)
10/5 (PT2) > p8 10/5 1-35 (patch regrowing with very mitotic areas, close, tight touching, non-uniform size, some large squamous)
10/11 (PT3) > p8 10/11 2-35 (OK so-so some good growing areas, some heavy patchy growth)
10/21 (PT4) > p8 10/21 2-35 (heavy condensed patch, so-so)

It was noted that 1-35 of p8 9/23, 1-35 p8 10/5 (PTed1) and 1-35 p8 10/21(PTed1) were discarded due to contamination. No record of fate of p8 10/11

p8 lineages:
p9 10/13 [p8 9/23 1-35 (PT1)] > 5-35 (good cft mitotic patches)
p9 10/18 [p8 9/23 1-35 (PT2) and 10/5 1-35 (PT1) (pool)] > 2-35 (good regrowth; good patchy growth)
p9 11/1 [p8 10/21 2-25 (PT1)] > 3-60, 1 T25 (some of these into MCDB 170)(almost cft good)

p9 12/2 [p8 9/23 1-35 (PT3)] > 1-35 (2 med and small cft OK patches on side of dish, so-so cells)
p9 12/7 [p8 9/23 1-35 (PT4)] > 1-35 (med size cft-overcft patch)
p9 12/14 [p8 9/23 1-35 (PT5)] > 1-35. Dish had yeast on 12/9. Washed, fed with 100% dosage fungizone. On 12/12 reduced to 1/4 fung. On 12/13 to 1/10. 12/14 there is yeast back. Wash extensively, PT refeeding with 1/10 fung. Record "also true for p9 12/5" (?12/7 dish with yeast and treated the same?)
p9 12/16 [p8 9/23 1-35 (PT6+)] > 3-35 A,B,C. Dish was good growing and yeast yesterday. Fed with 100% fung. Today questionable if yeast but cells not so happy. PT 3 separate times > A,B,C dishes (was trying to get rid of yeast which might be stuck to outsides of cells)
p9 12/22 [p8 9/23 1-35 (PT7+)] > 2-35. Yeast still present, extensively wash. PT 2 separate times
p9 1/3/84 [p8 9/23 1-35 (T8) fully trypsinize mother dish] > 2 T-25 and freezedown (**AA535**)

It was noted that 5-35 of p9 10/13 (i.e., all of PT1), 1-35 p9 12/2 (i.e., all of PT3) and 1-35(C) p9 12/16 (PT6; after 1 PT), 1-35 p9 10/18 (PT2; after 4 PT), 1-35 p9 12/9 (PT4; after 2 PT) were discarded due to contamination. No record of fate of progeny of p9 9/23 PT7 and PT8

p9 lineages:
<u>from p8 9/23 PT2</u>
p10 11/2 [p9 10/18 2-35 (PT1)] > 2-60, 2-35+(subcft good patchy growth)
p10 11/4 [p9 10/18 2-35 (PT2)] > 1 T25, 1-35, 3-60 (some end up in 170)(great looking heavy areas)
p10 11/14 [p9 10/18 2-35 (PT3)] > 1-60, 1-35 (few heavy cft tightly packed areas)
p10 11/18 [p9 10/18 2-35 (PT4)] > 2 T25 (med size tightly packed colonies getting heavy in center, growing at edges)
<u>from p8 10/21 PT1</u>
11/15 [p9 11/1 1-60] > freezedown (**AA506**) (OK subcft)
p10 11/16 [p9 11/1 1-60] > 4-35 (OK subcft)
[p9 11/1 1T25] > 4-60, 1-35 (OK subcft)
<u>from p8 9/23 PT4</u>
p10 12/19 [p9 12/9 (?12/7) 1-35 (PT1)] > 35s [notes unclear; I think various levels of fung. A,B,C ?-35 each and 3-35 into 170](good heavy packed patches ~65% of surface. Low level yeast still present, wash)
p10 12/22 [p9 12/9 (?12/7) (PT2x2)] > 1-35 each A,B 1/4 fung (patches regrowing still yeast present wash again 3X)
<u>from p8 9/23 PT5 and PT6</u>
p10 12/28 [p9 12/14 1-35 (PT1); p9 12/16 1-35(B) yeast free, pool] > 2T25, 1-60, 4-35 (~40% cft patches)
1/2/84 [p9 12/16 1-35(C) pooled with p10 12/22 1-35(B)] > freezedown (**AA528**) (tight patch growth)
p10 1/3 [p9 12/14 1-35 (PT2), p9 12/16 1-35(B) (PT2), p9 12/16 1-35(A) pool] > 1-35 and freezedown (**AA534**) (tight patch ~40-50% cft)

The following were noted as discarded due to no growth: p10 11/2 1-60, 1-35; p10 11/16 1 T-25, 3-35; p10 11/18 1 T-25. The following were discarded due to contamination or not listed why: ; p10 11/14 2-60; p10 11/16 2-35, 2-60 (from 170); p10 12/19C) 3-35 (after 1 PT); p10 12/22 1-T25 (after 1 PT); p10 1/2 1-60, 1-35

p10 lineages:
p11 12/21 [p10 11/2 1-35+ had coverglass but no yeast] > 3-35 (2 good heavy growing areas. Remove floating cells and seed directly 1-35; PT1 and seed 2-35, called "O")
12/22 [p10 12/19 refed yesterday without fung. today all clear] >
p11 12/28 [p10 12/19(B) 2-35 ~50% cft patches PT1] > 1-T25, 3-60, 1-35+
[p10 12/19(C) 3-35 yeast free, dense patchy growth 50% cft PT1] > 1-T25, 2-60, 3-35+
p11 12/30 [p10 11/2+ 2 main gr. patches and lots floating PT2] > 35s
[p10 12/19(C)+ 3-35 good regrow. ~50% cft PT2] > freezedown (**AA526**)
[p10 12/22(A) good dense patchy growth >60% cft 1-35, 1-T25 PT] > freezedown (**AA525**)
1/3/84 [p10 12/19(A) 1-35 and 12/19(B)+ 2-35 tight patch ~50% cft, pool] > freezedown (**AA533**)
p11 1/4 [p10 12/19(A+B++) 2-35 OK cft patches PT] > T25, 4-60, 3-25+, ?170 too
p11 1/19 [p10 1/3 heavy cft OK] > 3-60
p11 1/19 [p10 12/28 2-35 good very cft] > 3-60
p11 1/19 [p10 1/3 2-35 heavy cft OK] > 3-60

p11 lineages:
p12 1/2/84 [p11 12/21(O) 2-35 good patchy growth but doesn't seem as dense tight patches and maybe some cells escaping from patches] > T25, 4-60, 2-35 [T25 contam 1/10]
p12 1/5 [p11 12/28 2-60 good cft] > 2-T75, 3-60, 6-35+ and freezedown (**AA538**)[T75s, 1-35 contam 1/6]
1/6 [p11 12/28 T25 good cft] >freezedown (**AA539**)
p12 1/12 [p11 1/4 T25 subcft very tight patches OK] > 2-T75, 1-35+
p12 1/19 [p11 1/3 2-35 heavy cft OK] > 3-60
p12 2/2 [p11 1/19 3-60 3-60 cft good patchy f] > freezedown (**AA569Z**)

p12 lineages:
p13 1/11 [p12 1/5 (-CT) 1-60 cft good] > 2T25, 3-60, 3-35+
[p12 1/5 (+CT) 1-60 cft good] > 3-60
(I appear to have split this culture to ±CT though no record when)
p13 1/13 [p12 1/5 T25 subcft tight patch] > 2-T75, 3-60
p13 1/19 [p12 1/12 2-T75 good cft prob. with bacteria] > 4-35 and freezedown (**AA556**)
p13 1/25 [p12 1/18 from 170 into MM-CT, 1-60 good cft] > 2-T75, 3-60
p13 1/27 [p12 1/18 from 170 into MM+CT, 2-35 good-OK, almost cft tight] > T75, 3-60

p13 lineages:
p14 1/13 [p13 1/2(O) 1-60 OK-good cft, low level bacteria] > 1-35+ and freezedown (**AA545**)
p14 1/19 [p13 1/13 3-60, 2-T75 good cft,] > 3-35 and freezedown (**AA554**)
1/26 [p13 1/11 2-60 good cft,] > 3-35 and freezedown (**AA563Z**)
p14 2/1 [p13 1/25 1-60 good cft patches] > 4-60, 1-35
2/2 [p13 1/25 2-60 2-T75 good cft patchy] > freezedown (**AA567Z**)
p14 2/2 [p13 1/27 (-CT) 1-60 good cft-subcft patchy] > 4-60, 1-35+
2/3 [p13 1/27 (-CT) 2-60, 1-T75 OK cft] >freezedown (**AA575Z**)

p14 lineages:
p15 2/8 [p14 2/1 (+CT) 1-60 good cft patches] >3-60, 5-35+
p15 2/9 [p14 2/2 (-CT) 2-60 cft patches] >3-60
2/9 [p14 2/1 (+CT) 3-60 cft patches] > freezedown (**AA585Z**)
2/10 [p14 2/2 (-CT) 2-60 cft patches good] > freezedown (**AA586Z**)

Further original lineages in MM-CT derive directly from these (freezedowns AA601(Z), AA608, AA621, AA641(Z), AA653, AA659 which is p28)

Original lineages in MCDB 170
see p10 12/19 in MM (from p9 12/9 which was from p8 9/23 PT4) which was seeded into MCDB 170
p11 1/15 ±IP
p12 1/18 ±IP
freezedown +IP 2/1 (**AA566**); -IP 2/3 (**AA574(Z)**)
p13 1/30 ±IP
freezedown +IP 2/13 (**AA590**)

Further original lineages in 170+IP derive directly from these (freezedowns AA603(Z), AA611(Z), AA651, AA657, AA664(Z), AA675(Z) etc up to last freezedown AB164 at p50)

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Dear Colleagues,

This is to let you know that I have set up my HMEC newsletter list so that anyone on this list can send out an Email message/query to everyone else on the list. If you do not wish to be on this Email mailing list, you can take your name off by sending a message to hmec-request@lbl.gov with the subject word "unsubscribe", or you can Email me directly (this may take longer). If there is anyone else at your institution that would like to be placed on the list, they can Email the same address with the subject word "subscribe" or Email me with their Email address.

I frequently receive requests from investigators about whether anyone has or is studying a certain problem, or where particular reagents may be obtained, or best methods to perform studies, etc. This network will allow these queries to be sent to a much wider audience (my knowledge is limited!).

To send or reply to a message to the mailing list, use the address hmec@lbl.gov.

I would also like to remind everyone of my HMEC web site at

http://www.lbl.gov/~mrgs. This contains everything (and more) that you want to know about the derivation and use of the HMEC culture system developed by myself and collaborators.

I encourage all of you using these HMEC systems to send information for this web site, or to update date me on the course of your experiments. Send this information directly to my Email address, mrgs@lbl.gov. I would especially appreciate receiving information about any studies done in the past few years utilizing cultures I have sent (my grant renewal is due this year, and this would be most helpful to me). Information can be sent as:
(1) Abstracts from papers or meetings. Please let me know the complete reference to published/in press/submitted papers using cells I have sent.
(2) Short summaries of completed studies or work in progress
(3) Reagents from your lab that you would be willing to make available to other investigators
(4) Procedures from your lab that may be helpful to other investigators

If you would like to have this information posted on the web site, please indicate. We will format it here for posting. I will set up topic categories (e.g., cell cycle and senescence; growth factors and hormones; transformation and carcinogenesis; mutagenesis and carcinogen metabolism) so you can indicate a topic area.

I welcome any suggestions or feedback.

Martha